

Relationship among the leptin-to-adiponectin ratio, systemic inflammation, and anisocytosis in well-controlled type 2 diabetic patients with atherosclerotic cardiovascular disease

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KEY WORDS

adiponectin,
atherosclerotic
cardiovascular disease,
inflammation, leptin,
red blood cell
distribution width

EDITORIAL

by López-Jaramillo
et al, see p. 381

ABSTRACT

BACKGROUND Previous studies have shown that red blood cell distribution width (RDW) is an independent predictor of poor prognosis in type 2 diabetes (T2D) and atherosclerotic cardiovascular disease (ASCVD). The mechanisms underlying increased anisocytosis in patients with T2D and confirmed ASCVD remain poorly understood.

AIMS We sought to evaluate the relationship among the leptin-to-adiponectin ratio, systemic low-grade inflammation, and RDW in optimally treated patients with T2D and established ASCVD.

METHODS A total of 68 patients, aged 47 to 85 years (mean [SD], 65.3 [6.8] years) and including 21 women (30.9%), were enrolled and grouped according to median RDW into those with RDW <13.5% (n = 33) and those with RDW ≥13.5% (n = 35).

RESULTS Patients with RDW ≥13.5% had a significantly higher median (interquartile range [IQR]) serum leptin-to-adiponectin ratio (1.7 [0.49–2.3] ng/μg vs 0.66 [0.31–1.25] ng/μg; $P = 0.04$) and median (IQR) tumor necrosis factor α levels (1.58 [1.42–1.97] pg/ml vs 1.39 [1.18–1.57] pg/ml; $P = 0.02$). There were no significant differences in the concentrations of other inflammatory markers. The leptin-to-adiponectin ratio ($r = 0.25$; $P = 0.04$) and levels of tumor necrosis factor α ($r = 0.32$; $P = 0.01$) and soluble intercellular adhesion molecule 1 ($r = 0.31$; $P = 0.01$) were positively correlated with RDW, which was confirmed by univariate linear regression analysis. A multivariable regression model, which included demographic, clinical, and laboratory data, showed that white blood cell count ($\beta = 0.25$; 95% CI, 0.05–0.45; $P = 0.01$), soluble intercellular adhesion molecule 1 levels ($\beta = 0.21$; 95% CI, 0.02–0.41; $P = 0.03$), and mean corpuscular hemoglobin concentration (MCHC), ($\beta = -0.48$; 95% CI, 0.67 to -0.28 ; $P < 0.001$) were independent predictors of RDW in our patients.

CONCLUSIONS In well-controlled patients with T2D and ASCVD, the RDW values are associated with leptin-to-adiponectin imbalance and selected inflammatory markers.

INTRODUCTION Type 2 diabetes (T2D) represents a serious threat to global public health, currently affecting an estimated 451 million people worldwide.^{1–5} Atherosclerotic cardiovascular diseases (ASCVDs), including coronary artery disease (CAD), cerebrovascular disease, peripheral artery disease, and aortic

atherosclerotic disease remain the leading cause of morbidity and mortality in patients with T2D.^{1–5}

Red blood cell distribution width (RDW) is a simple measure of red blood cell (RBC) size heterogeneity (ie, anisocytosis), which is calculated by dividing the standard deviation (SD) of

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Received: December 30, 2019.
Revision accepted:
March 24, 2020.

Published online: March 24, 2020.
Kardiol Pol. 2020; 78 (5): 420–428
doi:10.33963/KP.15257
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WHAT'S NEW?

There is accumulating evidence that red blood cell distribution width (RDW), reflecting the degree of anisocytosis *in vivo*, is an independent predictor of poor prognosis in type 2 diabetes (T2D) and atherosclerotic cardiovascular disease (ASCVD). To the best of our knowledge, this is the first study to show that in patients with well-controlled T2D and established ASCVD, the RDW values are associated with leptin-to-adiponectin imbalance. Furthermore, a significant relationship was found between RDW and selected inflammatory markers. We believe that these findings will help understand the mechanisms underlying increased anisocytosis in patients with T2D and ASCVD as well as improve knowledge of its association with worse prognosis in this patient population.

RBC volumes by the mean corpuscular volume (MCV).⁶⁻¹² There is increasing evidence that anisocytosis measured by RDW values is increased in patients with T2D and ASCVD.⁶⁻¹² Furthermore, recent studies have shown that RDW is an independent predictor of poor prognosis in subjects with T2D and ASCVD.¹²⁻¹⁴ The mechanisms underlying increased anisocytosis in patients with T2D and confirmed ASCVD remain poorly understood. The results of experimental and clinical studies indicate a possible association of adipose tissue and adipokines, including leptin, adiponectin, interleukin 6 (IL-6), and tumor necrosis factor α (TNF- α), with hematopoiesis.¹⁴⁻²⁰ There are preliminary data suggesting that proinflammatory leptin, whose receptors have been found on the surface of hematopoietic cells, and anti-inflammatory adiponectin may be particularly important for hematopoiesis and possibly affect anisocytosis in both diabetic and nondiabetic patients.¹⁵⁻¹⁸

The aim of this study was to evaluate the relationship among the leptin-to-adiponectin ratio, systemic low-grade inflammation, and anisocytosis in optimally treated patients with T2D and established ASCVD.

METHODS Subjects The study included 126 consecutive outpatients who had T2D and angiographically documented CAD, peripheral artery disease, or extracranial artery disease. The exclusion criteria were as follows: type 1 diabetes, poorly controlled T2D with hemoglobin A_{1c} (HbA_{1c}) levels higher than 9%, a history of coronary intervention or coronary artery bypass grafting (≤ 1 month), a history of acute coronary syndrome (≤ 3 months), moderate-to-severe anemia (hemoglobin levels < 10 g/dl), treatment with iron, acute infection, hypertriglyceridemia, liver injury, chronic therapy with nonsteroidal anti-inflammatory drugs (except for acetylsalicylic acid), pregnancy, alcohol or drug abuse, life expectancy less than 12 months due to any concomitant diseases, and abnormal results of laboratory tests or imaging that would hamper the interpretation of findings. Finally, 68 patients with well-controlled T2D

and established ASCVD were enrolled. The median (interquartile range) duration of diabetes was 10 (6–15) years. Diabetic patients were grouped according to the median value of RDW into those with RDW $< 13.5\%$ ($n = 33$) and those with RDW $\geq 13.5\%$ ($n = 35$). Obesity was defined as body mass index higher or equal to 30 kg/m². The assessment of body composition was performed using bioelectrical impedance analysis.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the local ethics committee (KBET/190/B/2012). Written informed consent was obtained from all participants.

Sample collection and laboratory tests

Blood samples (25 ml) were obtained from the antecubital vein between 8 AM and 10 AM after an overnight fast. They were processed 30 to 60 minutes after blood collection. Then, serum samples were stored at -70°C until further analysis. Complete blood count, including RBCs, hemoglobin, hematocrit, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RDW, white blood cells (WBCs), neutrophils, lymphocytes, platelet count, and mean platelet volume, was assayed using the hematological analyzer Sysmex XT2000i (Sysmex Corporation, Kobe, Japan). Anemia was defined according to the WHO guidelines as blood hemoglobin concentration below 12 g/dl in women and below 13 g/dl in men.²¹

Routine blood tests, including lipid profile (total cholesterol, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], and triglycerides [TG]) and serum creatinine concentration, were performed using automated laboratory techniques. An estimated glomerular filtration rate (eGFR) was calculated by the abbreviated Modification of Diet in Renal Disease (MDRD) Study equation. The levels of HbA_{1c} were determined using a direct turbidimetric inhibition immunoassay.

Latex-enhanced nephelometry (Dade Behring, Marburg, Germany) was used for the measurement of fibrinogen and high-sensitivity C-reactive protein (hs-CRP) levels. The concentrations of IL-6, TNF- α , soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), and myeloperoxidase were measured by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota, United States).

Serum leptin and adiponectin levels were determined using radioimmunoassay kits (DIAsource, Louvain-la-Neuve, Belgium). The activity of lipoprotein-associated phospholipase A2 (Lp-PLA2) was measured with a colorimetric activity method assay (diaDexus, Inc., San Francisco, California, United States), in which

1-myristoyl-2-(4-nitrophenylsuccinylphosphatidylcholine) was used as a substrate.

The atherogenic index of plasma was calculated as $\log(\text{TG}/\text{HDL-C})$, with TG and HDL-C expressed in molar concentrations, as described elsewhere.²²

Statistical analysis Data were expressed as numbers and percentages for categorical variables and as mean (SD) or median (interquartile range) for the continuous variables, depending on their distribution. The Shapiro–Wilk test was used to determine normal distribution among continuous variables. Differences between the groups were assessed using the *t* test for normally distributed continuous variables or by the Mann–Whitney test for nonnormally distributed variables. The Pearson χ^2 test or the Fisher exact test (when any expected cell frequency was less than 5) were used to evaluate the differences in categorical variables between the respective study groups. Correlations were calculated with the Pearson coefficient. Stepwise linear regression analysis was performed to determine the independent predictors of RDW in the study patients. The final multivariable model included variables that were significant univariate predictors and did not exhibit significant collinearity. Two-sided *P* values less than 0.05 were

considered significant. All calculations were performed using the Statistica 13.3 software package (StatSoft, Inc., Tulsa, Oklahoma, United States).

RESULTS Baseline characteristics Finally, 68 patients aged 47 to 85 years (mean [SD], 65.3 [6.8] years) and including 21 women (30.9%) were enrolled. The baseline characteristics of the study patients are shown in TABLE 1. There were no intergroup differences in baseline demographics. Regarding clinical characteristics, only a history of percutaneous coronary interventions was more frequent in the group with $\text{RDW} \geq 13.5\%$ compared with the group with $\text{RDW} < 13.5\%$ (77.1 vs 51.5%; *P* = 0.03) (TABLE 1).

Patients with $\text{RDW} \geq 13.5\%$ had significantly lower mean (SD) levels of hemoglobin (13.2 [1.3] g/dl vs 13.9 [1.3] g/dl; *P* = 0.02), MCH (28.9 [1.8] pg vs 29.8 [1.3] pg; *P* = 0.01), and MCHC (33.2 [0.9] g/dl vs 34.1 [0.8] g/dl; *P* < 0.001) compared with those with $\text{RDW} < 13.5\%$ (TABLE 2). Anemia was diagnosed in 17.6% of the study patients. There were no differences in its prevalence between the studied groups (12.1% vs 22.9%; *P* = 0.25). In addition, subjects with higher RDW had decreased HDL-C levels (1.05 [0.8–1.35] mmol/l vs 1.26 [1.11–1.54] mmol/l; *P* = 0.03). The groups did not differ in HbA_{1c} levels (TABLE 2).

TABLE 1 Baseline characteristics of the study population

Variable	Whole group (n = 68)	RDW <13.5% (n = 33)	RDW ≥13.5% (n = 35)	<i>P</i> value
Age, y, mean (SD)	65.3 (6.8)	64.7 (7.2)	65.9 (6.4)	0.46
Female sex, n (%)	21 (30.9)	9 (27.3)	12 (34.3)	0.53
Hypertension, n (%)	66 (97.1)	31 (93.9)	35 (100)	0.23
Hyperlipidemia, n (%)	47 (69.1)	23 (69.7)	24 (68.6)	0.92
Metabolic syndrome, n (%)	68 (100)	33 (100)	35 (100)	>0.99
Obesity, n (%)	44 (64.7)	22 (66.7)	22 (62.9)	0.74
Waist circumference, cm, mean (SD)	106.5 (9.8)	104.7 (8.7)	108 (10.6)	0.19
BMI, kg/m ² , mean (SD)	31.1 (3.6)	31 (3.5)	31.2 (3.9)	0.85
Body fat, %, mean (SD)	33.8 (8.4)	34.4 (7.9)	33.3 (9)	0.61
Visceral fat, %, mean (SD)	16.1 (4.8)	16.4 (4.2)	15.5 (5.7)	0.52
Total body water, %, median (IQR)	47.5 (44.3–49.3)	47.6 (44.3–49)	46.3 (43.7–49.8)	0.9
Muscle mass, kg, mean (SD)	54.9 (10.5)	55.1 (9.8)	54.8 (10.5)	0.93
Medical history				
T2D duration, y, median (IQR)	10 (6–15)	9.5 (4–13)	10 (6–15)	0.71
CAD, n (%)	68 (100)	33 (100)	35 (100)	>0.99
PAD, n (%)	23 (33.8)	10 (30.3)	13 (37.1)	0.55
Previous MI, n (%)	27 (39.7)	11 (33.3)	16 (45.7)	0.3
Previous PCI, n (%)	44 (64.7)	17 (51.5)	27 (77.1)	0.03

Abbreviations: BMI, body mass index; CAD, coronary artery disease; IQR, interquartile range; MI, myocardial infarction; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; RDW, red blood cell distribution width; T2D, type 2 diabetes

TABLE 2 Hematological and glycometabolic parameters in the study patients

Variable	Whole group (n = 68)	RDW <13.5% (n = 33)	RDW ≥13.5% (n = 35)	P value
RBC, × 10 ⁶ /μl	4.6 (0.4)	4.7 (0.4)	4.6 (0.4)	0.4
Hemoglobin, g/dl	13.6 (1.3)	13.9 (1.3)	13.2 (1.3)	0.02
Hematocrit, %	40.4 (3.7)	40.9 (3.6)	39.8 (3.8)	0.23
MCV, fl	87.2 (3.8)	87.6 (3.1)	86.9 (4.4)	0.49
MCH, pg	29.3 (1.6)	29.8 (1.3)	28.9 (1.8)	0.01
MCHC, g/dl	33.6 (1)	34.1 (0.8)	33.2 (0.9)	<0.001
WBC, × 10 ³ /μl, median (IQR)	7.4 (6.4–9.1)	7.2 (6.4–7.9)	7.4 (6.4–9.8)	0.17
Neutrophils, × 10 ³ /μl, median (IQR)	4.4 (3.4–5.6)	4.4 (3.4–5.2)	4.7 (3.3–6.3)	0.39
Lymphocytes, × 10 ³ /μl, median (IQR)	2.1 (1.7–2.6)	2.1 (1.7–2.5)	2.2 (1.8–2.7)	0.15
Platelets, × 10 ³ /μl, median (IQR)	221 (174.5–277)	220 (203–252)	222 (154–310)	>0.99
MPV, fl	10.5 (1.3)	10.7 (1)	10.4 (1.5)	0.28
TC, mmol/l	3.85 (0.9)	4 (0.95)	3.71 (0.83)	0.19
LDL-C, mmol/l, median (IQR)	1.91 (1.53–2.69)	2.2 (1.63–2.85)	1.79 (1.45–2.47)	0.14
HDL-C, mmol/l, median (IQR)	1.22 (0.93–1.44)	1.26 (1.11–1.54)	1.05 (0.8–1.35)	0.03
TG, mmol/l, median (IQR)	1.4 (0.99–1.93)	1.32 (1.05–1.75)	1.47 (0.96–2.09)	0.44
AIP	0.1 (0.3)	0.04 (0.25)	0.15 (0.34)	0.14
HbA _{1c} , %, median (IQR)	7 (6.6–7.45)	6.9 (6.6–7.5)	7 (6.6–7.3)	0.93
Insulin, mU/ml, median (IQR)	20.6 (13.1–33)	18.87 (12.5–28.6)	22.1 (16.3–33.2)	0.49
C-peptide, ng/ml	3.3 (1.43)	2.99 (1.29)	3.6 (1.52)	0.08
Creatinine, μmol/l	84.4 (19.1)	84.3 (18.1)	84.5 (20.1)	0.97
eGFR, ml/min/1.73 m ² , median (IQR)	78.3 (68.5–90)	78.0 (72.5–88)	85.00 (67–90)	0.49

Data are given as mean (SD) unless otherwise indicated.

Abbreviations: AIP, atherogenic index of plasma; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, red blood cell; TC, total cholesterol; TG, triglycerides; WBC, white blood cell; others, see [TABLE 1](#)

TABLE 3 Adipokines and inflammatory markers in the study patients

Variable	Whole group (n = 68)	RDW <13.5% (n = 33)	RDW ≥13.5% (n = 35)	P value
Leptin, ng/ml	3.57 (1.71–7.65)	3.19 (1.16–7.15)	3.7 (2.25–9)	0.15
Adiponectin, μg/ml	3.73 (2.74–4.88)	4 (3.13–5.69)	3.44 (2.64–4.15)	0.13
Leptin-to-adiponectin ratio, ng/μg	0.93 (0.36–2.09)	0.66 (0.31–1.25)	1.7 (0.49–2.31)	0.04
hs-CRP, mg/l	1.52 (0.72–2.68)	1.66 (0.72–2.59)	1.43 (0.82–2.73)	0.97
IL-6, pg/ml	1.95 (1.56–2.76)	1.88 (1.46–2.34)	2.14 (1.62–3.19)	0.19
TNF-α, pg/ml	1.48 (1.27–1.77)	1.39 (1.18–1.57)	1.58 (1.42–1.97)	0.02
Myeloperoxidase, ng/ml	31.57 (19.22–48.2)	33.12 (22.76–52.88)	31.11 (14–47.22)	0.31
sICAM-1, ng/ml	211.90 (169.12–250.08)	198.96 (152.75–231)	224.83 (183.18–290.65)	0.07
sVCAM-1, ng/ml	983.06 (716.97–1504.74)	1138.24 (684.56–1545.27)	944.79 (730.76–1373.93)	0.54
Lp-PLA2, ng/ml, mean (SD)	138.53 (62.41)	136.88 (72.3)	140.1 (52.44)	0.83

Data are given as median (IQR) unless otherwise indicated.

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; Lp-PLA2, lipoprotein-associated phospholipase A2; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; TNF-α, tumor necrosis factor alpha; others, see [TABLE 1](#)

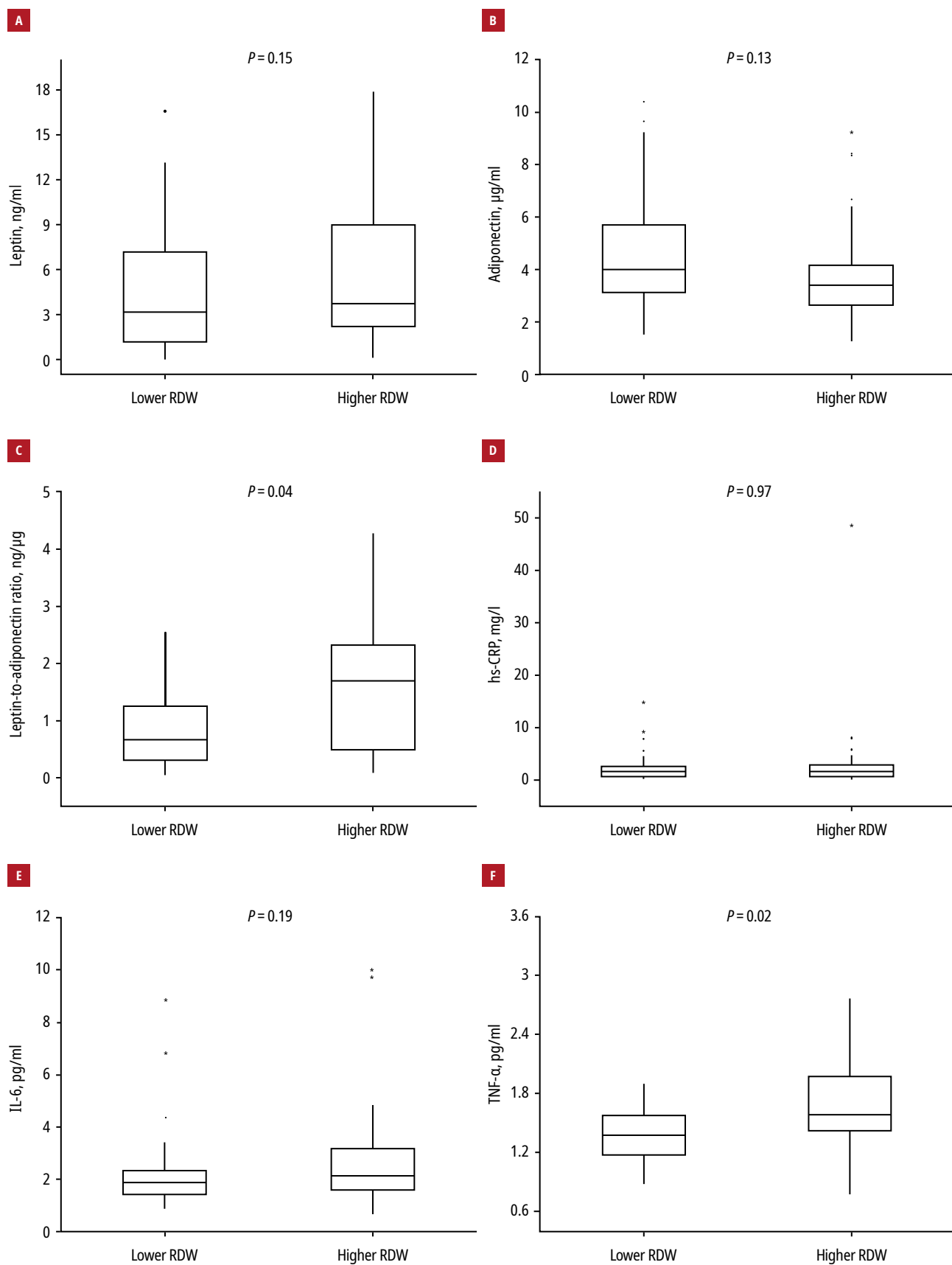


FIGURE 1 Distribution of adipokines and inflammatory markers in patients with lower (<13.5%) and higher ($\geq 13.5\%$) red blood cell distribution width (RDW): **A** – leptin; **B** – adiponectin; **C** – leptin-to-adiponectin ratio; **D** – high-sensitivity C-reactive protein (hs-CRP); **E** – interleukin 6 (IL-6); **F** – tumor necrosis factor α (TNF- α). Boxes represent medians and interquartile ranges; whiskers, the highest and lowest values; dots, outliers >1.5 interquartile ranges above the upper quartile; and asterisks, the extreme values.

TABLE 4 Linear regression analysis of red blood cell distribution width in the study patients

Variable	Unadjusted model		Adjusted model ^a	
	β (95% CI)	P value	β (95% CI)	P value
WBC, $\times 10^3/\mu\text{l}$	0.43 (0.21–0.66)	<0.001	0.44 (0.22–0.67)	<0.001
Neutrophils, $\times 10^3/\mu\text{l}$	0.35 (0.11–0.58)	0.005	0.35 (0.11–0.59)	0.005
Platelets, $\times 10^3/\mu\text{l}$	0.33 (0.1–0.56)	0.006	0.35 (0.12–0.59)	0.004
TNF- α , pg/ml	0.3 (0.06–0.53)	0.013	0.30 (0.06–0.54)	0.015
sICAM-1, ng/ml	0.3 (0.07–0.54)	0.012	0.33 (0.09–0.57)	0.008
History of PCI	0.26 (0.03–0.5)	0.029	0.26 (0.03–0.51)	0.033
Leptin-to-adiponectin ratio, ng/ μg	0.25 (0.01–0.48)	0.044	0.24 (0.01–0.49)	0.048
Lymphocytes, $\times 10^3/\mu\text{l}$	0.23 (–0.01 to 0.48)	0.062	0.26 (0.01–0.52)	0.043
MCV, fl	–0.22 (–0.46 to 0.02)	0.077	–0.25 (–0.5 to 0.01)	0.056
Hemoglobin, g/dl	–0.23 (–0.47 to 0.01)	0.055	–0.33 (–0.61 to –0.05)	0.02
MPV, fl	–0.36 (–0.59 to –0.13)	0.003	–0.36 (–0.59 to –0.13)	0.003
MCH, pg	–0.46 (–0.68 to –0.24)	<0.001	–0.47 (–0.69 to –0.25)	<0.001
MCHC, g/dl	–0.57 (–0.77 to –0.36)	<0.001	–0.61 (–0.83 to –0.4)	<0.001

a Adjusted for age and sex

Abbreviations: see TABLES 1–3

Leptin-to-adiponectin ratio and inflammatory markers Our study showed that patients with RDW $\geq 13.5\%$ had significantly higher serum leptin-to-adiponectin ratio (1.7 [0.49–2.31] ng/ μg vs 0.66 [0.31–1.25] ng/ μg ; $P = 0.04$) and TNF- α levels (1.58 [1.42–1.97] pg/ml vs 1.39 [1.18–1.57] pg/ml; $P = 0.02$) (TABLE 3 and FIGURE 1). There were no significant differences in the concentrations of other inflammatory markers (TABLE 3).

Correlations We found that leptin-to-adiponectin ratio ($r = 0.25$; $P = 0.04$) and the levels of TNF- α ($r = 0.32$; $P = 0.01$) and sICAM-1 ($r = 0.31$; $P = 0.01$) were positively correlated with RDW. No significant associations were observed between RDW and the levels of leptin and adiponectin. Furthermore, other inflammatory markers, such as hs-CRP and IL-6, were also not significantly related to the RDW. On the other hand, the significant correlations were found between circulating leptin and IL-6 levels ($r = 0.28$; $P = 0.02$) and between the leptin-to-adiponectin ratio and IL-6 levels ($r = 0.31$; $P = 0.01$).

Linear regression analysis The significant predictors of RDW are presented in TABLE 4. After adjusting for age and sex, WBC, neutrophils, lymphocytes, serum TNF- α levels, sICAM-1 levels, and the leptin-to-adiponectin ratio remained significantly associated with RDW (TABLE 4). A multivariable linear regression analysis, which included demographic, clinical, and laboratory parameters, showed that WBC count ($\beta = 0.25$; 95% CI, 0.05–0.45; $P = 0.01$),

sICAM-1 levels ($\beta = 0.21$; 95% CI, 0.02–0.41; $P = 0.03$), and MCHC ($\beta = -0.48$; 95% CI, –0.67 to –0.28; $P < 0.001$) were independent predictors of RDW in our patients.

DISCUSSION To our knowledge, this is the first study to show that anisocytosis is associated with leptin-to-adiponectin imbalance in patients with very high cardiovascular risk, T2D, and established ASCVD. Furthermore, a relationship was found between RDW and selected inflammatory markers.

RDW is a simple, inexpensive, and commonly available hematological parameter, which reflects the degree of anisocytosis in vivo.²³ In everyday clinical practice, RDW is most often used for the differential diagnosis of anemias.^{10,14,24–26} Importantly, there is emerging evidence suggesting a link between increased RDW and poor outcomes in the general population,^{27,28} in patients with CAD,^{29–31} and those with T2D and heart failure.¹⁴ Additionally, a significant relationship was found between RDW and the prevalence of T2D,^{12,13} metabolic syndrome,³² and heart failure.³³ These associations were independent of other hematological parameters including MCV, hemoglobin concentration, and hematocrit levels.¹⁴ Furthermore, in patients with chronic coronary syndromes, increased RDW was related to greater comorbidity burdens (ie, T2D, chronic kidney disease, atrial fibrillation, peripheral artery disease, and heart failure).⁷ Of note, increased RDW was associated with impaired microvascular perfusion and

could cause tissue hypoxia in patients with CAD, even in those without anemia.⁷

It is well documented that RDW increases under conditions of enhanced or ineffective RBC production (erythropoiesis) as well as enhanced RBC destruction.^{14,34} The pathophysiological mechanisms underlying increased RDW in patients with T2D and ASCVD are complex and not fully understood. A number of potential mechanisms have been proposed including harmful effects of hyperglycemia on RBCs (ie, rearrangement of RBC membranes, changes in the mechanical properties of cell membranes and cell metabolism, disturbance of oxygen binding to hemoglobin), bone marrow depression, chronic inflammation and related dysregulation of iron homeostasis, oxidative stress, and nutritional deficiencies (eg, of vitamin B₁₂, folic acid, or iron).¹⁴

Adipokine imbalance and anisocytosis

During erythropoiesis, numerous transcription factors, chromatin modifiers, cytokines, and hormones stimulate the proliferation and maturation of erythroid progenitors.^{22,35} In the present study, we found that serum leptin-to-adiponectin ratio was associated with RDW, a quantitative measure of anisocytosis. There is growing evidence that adipose tissue produces and actively secretes a plethora of biologically active adipokines that may disturb erythropoiesis by interacting with various metabolic and inflammatory pathways.^{16,20} In this context, it is very interesting that adipocytes account for about 50% of bone marrow cells and occupy 70% of adult bone marrow volume.^{15,36}

Leptin is a 16-kDa peptide hormone, produced predominantly by mature adipocytes, which is involved in regulating body energy balance and body fat.¹⁶ In adults, serum leptin levels are correlated with the percentage of body fat.¹⁶ Although the main role of leptin is to regulate body weight through its anorectic effect on the hypothalamus, this adipokine also has other functions, including stimulation of hematopoiesis and modulation of immune homeostasis.¹⁶ The leptin receptor (LEP-R) has been shown to be present at the cell membranes of hematopoietic cells including hematopoietic progenitor cells and erythropoietic, lymphoblastic, and myeloid cell lines.^{16,18} Studies on in vitro and animal models confirmed the key role of leptin at the early stages of erythropoiesis.³⁵ In addition to significant direct proliferative effects on erythroid precursor cells, leptin may also stimulate the differentiation of cells of erythrocytic lineage.¹⁶ Furthermore, it has been demonstrated that leptin acts synergistically with erythropoietin to promote erythropoiesis.^{15,16}

In turn, adiponectin is an adipocyte-derived hormone whose serum levels are negatively regulated by accumulation of visceral fat.³⁷ Adiponectin has anti-inflammatory, antidiabetic, and

antiatherogenic properties.³⁷ An increasing body of evidence suggests that hypoadiponectinemia is involved in the pathogenesis of T2D, CAD, and hypertension.³⁷ Adiponectin has been shown to enhance the proliferation of hematopoietic stem cells and maintain their undifferentiated state.³⁶

The results of our study showed that the leptin-to-adiponectin ratio was significantly related to the RDW values. This relationship was relevant after adjusting for age and sex. However, after considering all demographic, clinical, and laboratory data, the leptin-to-adiponectin ratio did not turn out to be an independent predictor of RDW in multivariable analysis. We postulate that this may reflect the significant yet not key role of this imbalance of adipokines in erythropoiesis in patients with well-controlled T2D and ASCVD.

As the leptin-to-adiponectin ratio is a well-established marker of adipose tissue dysfunction, strongly associated with the surrogate measures of insulin resistance such as the homeostatic model assessment (HOMA), the hyperinsulinemic-euglycemic clamp, and the quantitative insulin sensitivity check index (QUICKI) in various cohorts,^{38,39} we hypothesize that RDW values can reflect the severity of adiposopathy and insulin resistance in diabetic patients with very high cardiovascular risk and documented ASCVD. Interestingly, Inoue et al⁴⁰ showed that the leptin-to-adiponectin ratio in individuals with T2D was correlated with insulin resistance even stronger than HOMA. It has also been suggested that an elevated leptin-to-adiponectin ratio may be a useful atherosclerotic index in patients with T2D.⁴¹ In addition, leptin-to-adiponectin imbalance was associated with increased vasoconstriction caused by angiotensin II.³⁷

Leptin-to-adiponectin ratio and chronic inflammation

Chronic systemic inflammation plays a crucial role in the pathogenesis of T2D and its macrovascular complications.¹⁻⁵ It is well known that low-grade inflammation is closely linked to all stages of ASCVD.^{42,43}

Leptin and adiponectin have opposing effects on systemic inflammation.³⁷ Leptin upregulates pro-inflammatory cytokines including IL-6 and TNF- α , which are associated with insulin resistance and T2D.³⁷ In contrast, adiponectin downregulates the expression of various proinflammatory mediators and exerts anti-inflammatory properties.³⁷ Our study is consistent with these observations, as we found a significant positive correlation between leptin and IL-6 levels as well as between the leptin-to-adiponectin ratio and IL-6 levels.

Anisocytosis and systemic inflammation

Chronic inflammation may influence erythropoiesis, including RBC circulatory half-life and RBC deformability, and therefore increase RDW

values.⁴⁴ A shortened RBC half-life was reported for T2D and metabolic syndrome and associated with increased anisocytosis.^{32,45} These mechanisms may explain the relationship between increased RDW and chronic subclinical inflammation, which is typical of diabetes. Recent studies have also shown that systemic inflammation may increase the degree of anisocytosis by inducing the expression of hepcidin, which inhibits intestinal iron absorption and iron release from macrophages.⁴⁶ Furthermore, it has been shown that proinflammatory cytokines can directly affect erythropoiesis by inhibiting the proliferation of erythroid progenitor cells and impairing the erythropoietin gene expression, which leads to increased RDW.^{46,47} It was found that TNF- α inhibits the growth of burst- and colony-forming unit-erythroid cells.⁴⁶ Previous studies have also shown that proinflammatory cytokines inhibit erythropoietin-induced RBC maturation.⁴⁶ Therefore, some researchers, yet not all, suggest that RDW may be a simple marker of systemic low-grade inflammation in various clinical entities including T2D.^{12-14,32,44,47} In line with these observations, we found a positive correlation of RDW with serum TNF- α and sICAM-1 (a marker of vascular inflammation) levels. Furthermore, univariate and multivariable linear regression analyses showed a significant association between selected inflammatory markers and RDW. Interestingly, we did not find any relationship between RDW and hs-CRP. There is strong evidence that both TNF- α and IL-6 induce the expression of hs-CRP in the liver and are more sensitive inflammatory markers than hs-CRP.⁴⁸ In addition, this may be due to the modulating effect of obesity on the relationship between chronic inflammation and anisocytosis in the study patients. Of note, obesity occurred in 64.7% of our patients and the mean (SD) body mass index was 31.1 (3.6) kg/m². Vayá et al⁴⁷ did not find a relationship between inflammatory markers and RDW in the morbidly obese patients, which may partly explain our results.

Chung et al¹⁷ demonstrated that leptin can directly regulate hepatic expression of hepcidin. It has been shown that increased hepcidin biosynthesis in the presence of leptin decreases duodenal iron absorption and impairs iron metabolism, which can lead to hypoferrremia.¹⁷ In the present study, we did not evaluate iron metabolism in our patients; however, the strong and independent inverse relationship between RDW and MCHC suggests that the pathomechanism proposed by Chung et al,¹⁷ rather than the direct impact of systemic inflammation, plays a key role in the mechanisms of anisocytosis in our study patients.

Study limitations This study had several limitations. First, our study, although prospective and dual-center, focused on a selected group of patients. Second, the sample size was relatively

small and a larger sample would probably provide more robust findings. Finally, investigating iron metabolism and hypoferrremia could help clarify the association between RDW, systemic inflammation, and the leptin-to-adiponectin ratio in patients with T2D and ASCVD.

Conclusions Our study showed that RDW values in patients with well-controlled T2D and ASCVD were associated with leptin-to-adiponectin imbalance and selected inflammatory markers.

ARTICLE INFORMATION

ACKNOWLEDGMENTS This work was supported by a research grant from the National Science Centre, Poland (2011/03/B/NZ5/0576; to GG).

CONFLICT OF INTEREST None declared.

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HOW TO CITE Rostoff P, Siniarski A, Haberka M, et al. Relationship among the leptin-to-adiponectin ratio, systemic inflammation, and anisocytosis in well-controlled type 2 diabetic patients with atherosclerotic cardiovascular disease. *Kardiol Pol.* 2020; 78: 420-428. doi:10.33963/KP.15257

REFERENCES

- 1 Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J.* 2020; 2: 255-323.
- 2 Cho NH, Shaw JE, Karuranga S, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018; 138: 271-281.
- 3 Gajos G. Diabetes and cardiovascular disease: from new mechanisms to new therapies. *Pol Arch Intern Med.* 2018; 128: 178-186.
- 4 Gajos G, Siniarski A, Natorka J, et al. Polyhydrocytes in blood clots of type 2 diabetic patients with high cardiovascular risk: association with glycemia, oxidative stress and platelet activation. *Cardiovasc Diabetol.* 2018; 17: 146.
- 5 Poreba M, Rostoff P, Siniarski A, et al. Relationship between polyunsaturated fatty acid composition in serum phospholipids, systemic low-grade inflammation, and glycemic control in patients with type 2 diabetes and atherosclerotic cardiovascular disease. *Cardiovasc Diabetol.* 2018; 17: 29.
- 6 Danese E, Lippi G, Montagnana M. Red blood cell distribution width and cardiovascular diseases. *J Thorac Dis.* 2015; 7: E402-E411.
- 7 Mozos I. Mechanisms linking red blood cell disorders and cardiovascular diseases. *Biomed Res Int.* 2015; 2015: 682054.
- 8 Li N, Zhou H, Tang Q. Red blood cell distribution width: a novel predictive indicator for cardiovascular and cerebrovascular diseases. *Dis Markers.* 2017; 2017: 7089493.
- 9 Szygula-Jurkiewicz B, Siedlecki Ł, Pyka Ł, et al. Red blood cell distribution width, relative lymphocyte count, and type 2 diabetes predict all-cause mortality in patients with advanced heart failure. *Pol Arch Intern Med.* 2018; 128: 115-120.
- 10 Eroglu E, Kilicgedik A, Kahveci G, et al. Red cell distribution width and its relationship with global longitudinal strain in patients with heart failure with reduced ejection fraction: a study using two-dimensional speckle tracking echocardiography. *Kardiol Pol.* 2018; 76: 580-585.
- 11 Salvatori M, Formiga F, Moreno-Gonzalez R, et al. Red blood cell distribution width as a prognostic factor of mortality in elderly patients firstly hospitalized due to heart failure. *Kardiol Pol.* 2019; 77: 632-638.
- 12 Nada AM. Red cell distribution width in type 2 diabetic patients. *Diabetes Metab Syndr Obes.* 2015; 8: 525-533.
- 13 Engström G, Smith JG, Persson M, et al. Red cell distribution width, haemoglobin A1c and incidence of diabetes mellitus. *J Intern Med.* 2014; 276: 174-183.
- 14 Xanthopoulos A, Giamouzis G, Melidonis A, et al. Red blood cell distribution width as a prognostic marker in patients with heart failure and diabetes mellitus. *Cardiovasc Diabetol.* 2017; 16: 81.
- 15 Stenvinkel P, Heimbürger O, Lönnqvist F, Bárány P. Does the *ob* gene product leptin stimulate erythropoiesis in patients with chronic renal failure? *Kidney Int.* 1998; 53: 1430-1431.
- 16 Kinik ST, Ozbek N, Yücel M, et al. Correlations among serum leptin levels, complete blood count parameters and peripheral CD34(+) cell count in prepubertal obese children. *Ann Hematol.* 2005; 84: 605-608.

- 17 Chung B, Matak P, McKie AT, Sharp P. Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. *J Nutr.* 2007; 137: 2366-2370.
- 18 Han TJ, Wang X. Leptin and its receptor in hematologic malignancies. *Int J Clin Exp Med.* 2015; 8: 19840-19849.
- 19 Vuong J, Qiu Y, La M, et al. Reference intervals of complete blood count constituents are highly correlated to waist circumference: should obese patients have their own "normal values?" *Am J Hematol.* 2014; 89: 671-677.
- 20 Coimbra S, Ferreira C, Belo L, et al. Impact of weight loss on inflammation and red blood cell biomarkers after laparoscopic gastric banding surgery. *J Investig Med.* 2018; 66: 304-308.
- 21 World Health Organization. Iron deficiency anaemia: assessment, prevention, and control. A guide for programme managers. World Health Organization; 2001.
- 22 Dobiášová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). *Clin Biochem.* 2001; 34: 583-588.
- 23 Lippi G, Turcato G, Cervellin G, Sanchis-Gomar F. Red blood cell distribution width in heart failure: a narrative review. *World J Cardiol.* 2018; 10: 6-14.
- 24 Kalemci S, Akin F, Sarihan A, et al. The relationship between hematological parameters and the severity level of chronic obstructive lung disease. *Pol Arch Intern Med.* 2018; 128: 171-177.
- 25 Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: a simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci.* 2015; 52: 86-105.
- 26 Weymann A, Ali-Hasan-Al-Saegh S, Popov AF, et al. Haematological indices as predictors of atrial fibrillation following isolated coronary artery bypass grafting, valvular surgery, or combined procedures: a systematic review with meta-analysis. *Kardiologia Pol.* 2018; 76: 107-118.
- 27 Perlstein TS, Weuve J, Pfeiffer MA, Beckman JA. Red blood cell distribution width and mortality risk in a community-based prospective cohort. *Arch Intern Med.* 2009; 169: 588-594.
- 28 Patel KV, Semba RD, Ferrucci L, et al. Red cell distribution width and mortality in older adults: a meta-analysis. *J Gerontol A Biol Sci Med Sci.* 2010; 65: 258-265.
- 29 Tonelli M, Sacks F, Arnold M, et al. Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease. *Circulation.* 2008; 117: 163-168.
- 30 Osadnik T, Strzelczyk J, Hawranek M, et al. Red cell distribution width is associated with long-term prognosis in patients with stable coronary artery disease. *BMC Cardiovasc Disord.* 2013; 13: 113.
- 31 Tsuboi S, Miyauchi K, Kasai T, et al. Impact of red blood cell distribution width on long-term mortality in diabetic patients after percutaneous coronary intervention. *Circ J.* 2013; 77: 456-461.
- 32 Sánchez-Chaparro MA, Calvo-Bonacho E, González-Quintela A, et al. Higher red blood cell distribution width is associated with the metabolic syndrome: results of the Ibermutuamur Cardiovascular Risk assessment study. *Diabetes Care.* 2010; 33: e40.
- 33 Huang YL, Hu ZD, Liu SJ, et al. Prognostic value of red blood cell distribution width for patients with heart failure: a systematic review and meta-analysis of cohort studies. *PLoS One.* 2014; 9: e104861.
- 34 Emans ME, van der Putten K, van Rooijen KL, et al. Determinants of red cell distribution width (RDW) in cardiorenal patients: RDW is not related to erythropoietin resistance. *J Card Fail.* 2011; 17: 626-633.
- 35 Al-Rubaie SM, Daoud MS, Al-Kadiri TE. Association of erythropoietin, adiponectin and leptin levels with anemia in uremic diabetic patients (under hemodialysis). *J Fac Med Baghdad* 2010; 52: 432-437.
- 36 Wang H, Leng Y, Gong Y. Bone marrow fat and hematopoiesis. *Front Endocrinol (Lausanne).* 2018; 9: 694.
- 37 López-Jaramillo P, Gómez-Arbeláez D, López-López J, et al. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. *Horm Mol Biol Clin Invest.* 2014; 18: 37-45.
- 38 Frühbeck G, Catalán V, Rodríguez A, et al. Involvement of the leptin-adiponectin axis in inflammation and oxidative stress in the metabolic syndrome. *Sci Rep.* 2017; 7: 6619.
- 39 Frühbeck G, Catalán V, Rodríguez A, Gómez-Ambrosi J. Adiponectin-leptin ratio: a promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. *Adipocyte.* 2018; 7: 57-62.
- 40 Inoue M, Maehata E, Yano M, et al. Correlation between the adiponectin-leptin ratio and parameters of insulin resistance in patients with type 2 diabetes. *Metabolism.* 2005; 54: 281-286.
- 41 Kotani K, Sakane N, Saiga K, Kurozawa Y. Leptin: adiponectin ratio as an atherosclerotic index in patients with type 2 diabetes: relationship of the index to carotid intima-media thickness. *Diabetologia.* 2005; 48: 2684-2686.
- 42 Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation.* 2002; 105: 1135-1143.
- 43 Sanak M, Plutecka H, Szczekliki W, et al. Functional promoter polymorphism of cyclooxygenase-2 modulates the inflammatory response in stable coronary heart disease. *Pol Arch Med Wewn.* 2010; 120: 82-88.
- 44 Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med.* 2005; 352: 1011-1023.
- 45 Acosta J, Hettinga J, Flückiger R, et al. Molecular basis for a link between complement and the vascular complications of diabetes. *Proc Natl Acad Sci USA.* 2000; 97: 5450-5455.
- 46 Gang L, Lifang W. Association of the elevated red blood cell distribution width with the risk of developing diabetes mellitus. *Intern Med.* 2016; 55: 1959-1965.
- 47 Vayá A, Alis R, Hernandez-Mijares A, et al. Red blood cell distribution width is not related with inflammatory parameters in morbidly obese patients. *Clin Biochem.* 2014; 47: 464-466.
- 48 Kim KI, Lee JH, Chang HJ, et al. Association between blood pressure variability and inflammatory marker in hypertensive patients. *Circ J.* 2008; 72: 293-298.